Ig G-, but also Ig A- and Ig M-class Ab, and which can be applied to seminal plasma (SP) samples. The clinical relevance during infertility investigation was tested in this prospective study.

Materials and Methods: A total of 173 males from randomly chosen subfertile couples without symptoms of genital tract infections (median duration of infertility 3.5 (range 1-19) years) were enrolled in the study (median age 33 (range 22-52) years). Their female partners were examined at the same time (median age 31 (range 21-43) years). Couples were not selected with regard to infertility factors (primary infertility 89.1% secondary infertility 10.9%). Serum of male patients and their partners and same-day seminal plasma samples were screened for anti-chlam. Ab of the Ig G-, Ig A- and Ig M- classes using a commercial recombinant enzyme-linked immunosorbent assay (ELISA) based on chlamydial lipopolysaccharide (LPS) fragment antigens. The outcome of chlamydial serology (with a total of nine samples per couple) was analyzed with regard to multiple parameters of semen quality (evaluated the same day): e.g. results of microscopic sperm analysis, semen cultures, seminal leukocytes (LC) as potential marker of silent infection/inflammation and sperm ability (under standardized in vitro conditions) to penetrate the cervical mucus (CM) barrier as a significant determinant of sperm functional capacity. Patients’ medical history and clinical andrological examination were taken into consideration as well as results of postcoital testing (PCT), and subsequent fertility (after control for female infertility factors).

Results(s): Anti-chlamydial Ab of the three Ig classes in serum of male patients were significantly interrelated (e.g. Ig M/Ig A Ab p < 0.01). Chlam Ig A class Ab in seminal fluid were significantly associated with findings in same-day serum (p < 0.001). Evidence for previous chlamydial infection was significantly more frequent in female partners of males with anti-chlam Ab in serum (p < 0.001), and also of partners of patients with Chlam Ig A Ab in their seminal plasma (p < 0.005). The outcome of chlamydial serology (Chlam Ig G-, Ig A- and Ig M-class Ab) in serum, as well as in same-day SP, was not significantly related to results of clinical examination and sperm quality, evaluated with standard semen analysis, seminal leukocytes, and semen cultures. There was no significant relationship of Chlam serology in both partners with the outcome of sperm-CM interaction in vitro and with results of PCT.

Conclusion(s): As part of a comprehensive investigation of male fertility, the outcome of chlamydial serology in serum and same-day seminal plasma was not indicative of semen quality and sperm functional capacity.
Materials and Methods: All RCTs of oral antioxidant supplements in men were searched in the following sources: the Cochrane Menstrual Disorders and Subfertility Group Register, MEDLINE, CENTRAL, EMBASE, CINAHIL, PSYCINFO and AMED databases (from their inception until January 2010), trial registers, unpublished literature, reference lists and experts in the field. RCTs comparing any type or dose of antioxidant (single or combined) versus placebo, no treatment or another antioxidant that were taken by the male partner of a couple seeking fertility assistance were included. The outcomes were live birth, pregnancy, miscarriage, or spontaneous abortion, stillbirth, level of sperm DNA damage, sperm motility, sperm concentration and adverse effects.

We performed statistical meta-analyses in accordance with the guidelines developed by The Cochrane Collaboration for the effect of antioxidant/s versus placebo per couple randomised.

Results: Fifty trials were considered and 32 met the inclusion criteria. 2696 couples in total.

Live birth: Two trials reported live birth. The use of antioxidants in men compared to placebo was associated with an increased live birth rate (pooled odds ratio (OR) 6.44, (1.72 to 24.04, 95% CI = 0%, p = 0.006)). This result was based on 10 live births from a total of 117 couples in the two studies. One of these trials included couples undergoing IVF.

Pregnancy rate: There were 79 pregnancies in 11 trials including 795 couples. Antioxidant use compared to placebo was associated with an increased pregnancy rate (pooled OR 3.89 (2.33 to 6.49, 95% CI = 0, p < 0.0001)). Sensitivity analysis on two trials that included couples undergoing IVF showed that the use of antioxidant remains associated with increased pregnancy rate (pooled OR 4.22, (2.33 to 7.63, 95% CI = 0%, p < 0.00001)).

Miscarriage rate: There was no evidence of an effect on miscarriage rates, (pooled OR 1.15 (0.21 to 6.28; p = 0.87)) between the antioxidant and placebo groups in two trials, 145 couples.

Still Birth: There were no trials reporting stillbirth.

DNA fragmentation: One trial reported DNA fragmentation. There was a difference (OR -13.80, (-17.50 to -10.10; p < 0.00001)) in favour of the antioxidant group over the placebo.

Total sperm motility: Antioxidant supplementation in men compared to placebo was associated with an improvement in total sperm motility for the following timeframes:

1. at ≤3 months: pooled OR 9.88 (7.17 to 12.59; 95% CI = 52%, p < 0.00001). 348 participants studied in seven trials.
2. at 6 months pooled OR 4.19 (3.81 to 4.56; 95% CI = 89%, p < 0.00001). 915 participants studied in seven trials.
3. at ≥9 months: pooled OR 1.38, (0.81 to 1.95; 95% CI = 64%, p < 0.00001). 332 participants in three trials.

Sperm concentration: Antioxidant use compared to placebo was associated with an improvement in sperm concentration within the following time frames:

1. at ≤3 months: There was no beneficial effect determined; pooled OR 2.64, (-0.52 to 5.81; p = 0.10), six trials of 290 participants.
2. at 6 months: pooled OR 5.25, (4.43 to 6.08; p < 0.00001; 95% CI = 53%), six trials of 825 participants.
3. at ≥9 months: pooled OR 1.61 (0.61 to 2.61; P = 0.002, 95% CI = 0%), three trials of 332 participants.

Side effects: No studies reported evidence of harmful side effects of the antioxidant therapy used.

Conclusions: There is some evidence that antioxidant supplementation in sub-fertile males may improve the outcomes of live birth, pregnancy rate and sperm parameters for subfertile couples.

P-007 Identification and isolation of a protein from human oviductal secretion that interact with human spermatozoa
C. Zumoffen1, M.J. Munuce2, A. Caille2, S. Ghersevich1
1School of Biochemical and Pharmaceutical Sciences, Laboratory of Reproductive Studies - Clinical Biochemistry, Rosario, Argentina

Introduction: After ejaculation, a number of spermatozoa move to the first portion of the oviduct, where they can remain viable during hours or even days in contact with the oviductal secretion and can undergo several metabolic and functional changes to become fertilizing-competitive (process known as capacitation). We have found that proteins from conditioned medium (CM) of human oviductal tissue cultures can modulate some sperm functions. It has been suggested that some oviductal proteins interact with human spermatozoa and could modulate sperm function. Thus, the aim of the present study was to isolate and identify proteins from CM with capacity to interact with human spermatozoa.

Materials and Methods: Human oviductal tissue was obtained from premenopausal women (age: 41.0 ± 1.6, n = 20) with no clinical history of infection or cancer disease, scheduled for routine hysterectomies. Native human oviductal fluid (nOF) was recovered by flushing the tubes with DMEM/Ham F12 medium. Explants of tubal tissues were cultured in DMEM/Ham F12 medium at 37 °C and 5 % CO2 for 24 h, followed by a further incubation with [35S]Met (30 μCi/ml) during 24 hs, to obtain de novo [35S]Met-proteins. After incubation, CM was collected and centrifuged 5 min at 700 × g to remove debris. The CM were then dialysed, lyophilised and stored at -70°C until use. Total protein concentration in CM was determined using the Bradford’s assay. Human spermatozoa were obtained from normozoospermic samples of healthy donors (n = 4) after 3 days of sexual abstinence. Motile sperm were recovered by swim-up and were incubated under capacitating conditions in Ham F10 medium supplemented with 3.5 % BSA for 2 h in the absence or the presence of [35S]Met-proteins from CM. At the end of incubations, sperm membrane proteins (SMP) were extracted, analysed by SDS-PAGE (10%) and electrophoretically transferred to nitrocellulose membranes. In addition, a chromatographic affinity column was prepared with motile sperm membrane extracts coupled to Sepharose 4B, in which CM was seeded. The eluted protein fractions were subjected to SDS-PAGE 10%. The protein bands were analysed by LC-MS/MS. The presence of the identified protein in nOF and CM was examined by Western blot analysis.

Results: A protein band with an estimated molecular weight (MW) of 14 kDa was detected in the autoradiographies of SMP from spermatozoa that were incubated with [35S]Met-labelled oviductal proteins. The SDS-PAGE of eluted protein fractions obtained by affinity chromatography showed the presence of at least five [35S]Met-protein bands by autodigestion, with estimated MW of 127 kDa, 94 kDa, 79 kDa, 17 kDa and 14 kDa, respectively. The 14 kDa protein was identified as human calgranulin B by LC-MS/MS analysis. The presence of calgranulin B in CM and nOF was confirmed by Western blot using specific antibodies.

Conclusion: At least five oviductal proteins that interact with sperm membrane were detected (127 kDa, 94 kDa, 79 kDa, 17 kDa and 14 kDa). In the protein extract from sperm incubated in the presence of CM a protein of approximately 14 kDa was also present. This protein was identified as human calgranulin B and it was detected both in CM and nOF by Western blot. These results support the hypothesis that some oviductal proteins could modulate the sperm function through direct binding on sperm and could have an active role in the fertilization process.

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P-008 Variations in folate pathway genes are associated with male infertility
A.M. Lendinez1, B. Perez-Nevo1, A.R. Palomares2, A. Serrano Garballo1, A. Rodriguez1, A. Reche1, A. Mayor-Olea1, M. Ruiz-Galdón1, A. Reyes-Engel1
1Hospital Clínico Universitario Virgen de la Victoria, Análisis Clínicos, Malaga, Spain
2Instituto de Fertilitad Clínica Rincón, Bioquímica y Biología Molecular, Malaga, Spain
3Hospital Clínico Universitario Virgen de la Victoria, Ginecología y Obstetricia, Malaga, Spain
4Hospital Materno Infantil. Carlos Haya. Ginecología y Obstetricia, Malaga, Spain
5Facultad de Medicina. Universidad de Málaga, Bioquímica y Biología Molecular, Malaga, Spain

Introduction: Folate gene polymorphisms have been previously related with reproduction disorders. Recently several association studies have suggested that polymorphic variants in the MTHFR gene may be associated with reduced sperm counts in the human leading to male infertility in some populations. In the present study we have analyzed 19 polymorphisms from 13 genes of folate cycle in infertile males.

Materials and Methods: A group of 28 infertile men (classified according to WHO and Kruger criteria) and 122 controls were genotyped for the following